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Research Article

Spelt (*Triticum aestivum* ssp. *Spelta*)– from Field to Cosmetics

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ABSTRACT

Plants have been intensively used as sources of biologically active compounds. Recently, the attention was directed to spelt (*Triticum aestivum* ssp. *spelta*) as a promising source of antioxidant compounds. In the present study, the antioxidant activity and total phenolic content of *Triticum spelta* glycerin extracts were determined. Three different extraction techniques were performed – conventional, microwave- and ultrasound- assisted extractions. Best polyphenol content and antioxidant activity were resulted when heat reflux extraction was applied. Moreover, nourishing and regenerating cream (NRC) formulation was prepared by incorporating 1 % glycerin spelt extract. The physicochemical stability of this formulation was assessed as well as sensory and skin penetration caracteristics.The cosmetic preparation exhibited good emulsion and colour stability at all temperature tested (-10 °C; 25 °C and 40 °C) during 90 days. 85 % of volunteers assessed the skin penetration of NRC as good. The same percentage claimed pleasant odor caracteristic after application on skin. Based on these results it can be concluded that spelt is a promissing source of biologically active substances with various applications.

Keywords: antioxidant activity, cosmetic application, spelt (*Triticum aestivum* ssp. *spelta*).

INTRODUCTION

Plants, as a primary source of bioactive compounds, produce a broad range of metabolites with diverse activities. A particular interest represents the production of flavonoids and other phenolics which are part of plant growth and defense against infection and injury[1]. The antioxidant activity of phenolics is a function of their reducing properties, hydrogen donation, and singlet oxygen quenching. Moreover, they have a metal chelation potential[2]. *Triticum* species are a good source of polyphenols[3]. Brandolini et al.[3] demonstrated that phenolic acid is rare in endosperm, but abundant in germ and bran. Incorporated in skin care cosmetics those compounds quench reactive oxygen species, including hydroxyl radicals, superoxide anions, and fatty peroxy radicals, to protect the skin from oxidative damage[4].

The extraction of bioactive compounds from plant materials is the first step in their characterization and utilization[5]. The recovery of plant antioxidant compounds is achieved through different extraction solvents and techniques. The antioxidant capacity of these extracts largely depend on the composition of the extracts, the nature of the solvent, and experimental conditions[6]. Polar solvents are frequently employed with aqueous ethanol and methanol being more efficiently used[7]. However, methanol is toxic and highly flammable, ethanol is expensive due to restrictions arising from state laws and therefore they are completely incompatible with a "green" extraction processs[8]. Moreover, cosmetic formulations are mainly emulsions that are not compatible with alcohol solvents. Glycerin is a natural, non-toxic and low-cost substance, largely used in skincare products. Glycerin in aqueous solutions can favorably change the polarity of water, and turning into efficient co-solvent for increased polyphenol extraction[8].

The term "natural" is defined as something or an ingredient that is produced by the nature or found in nature and is directly extracted from plants or animal products[9]. The incorporation of plant extracts in products has been highlighted by consumer demand, who are increasingly concerned with buying ecologically friendly products[9,10]. Cosmetic and cosmeceutical preparations from herbal origin are typically nontoxic and possess strong antioxidant activity[11].

Spelt wheat (*Triticum aestivum* ssp. *spelta*) is one of the oldest crop grown in Europe[12]. It belongs to the so called "ancient" wheat species. Compared to wheat (*Triticum aestivum* L.), spelt is more environmentally hardy, survives in cool and wet conditions and requires lower nitrogen fertilization levels as well[12,13]. Moreover, spelt can be grown on poorly-drained and low-fertility soils[10]. Although, the cultivation of spelt has decreased during the last years, recent interest for ecologically grown foods has led to resurgence in its cultivation[14]. Spelt wheat is

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rich in oleic and linoleic fatty acids[15] and has a good proportion of soluble fibres, moreover the starch is more rapidly hydrolysed[16]. Compared to wheat spelt has a higher protein and lipid content and a lower insoluble and total fiber content[17]. It could be used in bread production as an additional flour[16].

The aim of the present study was to obtain and compare extracts of *Triticum spelta* in respect of total polyphenolic content and *in vitro* antioxidant activity. Different extraction techniques were conducted with glycerin:water as solvent. The extract with the highest biological activity was incorporated in cosmetic formulation. Then the cosmetic stability and volunteer acceptance was asseced.

MATERIALS AND METHODS

Triticum aestivum ssp. *spelta* grains were bought from a local health food store (Plovdiv, Bulgaria), roughly grounded and stored in air-tight dark containers until extraction.

Folin Ciocalteu reagent, Na_2CO_3, gallic acid, ABTS, $CuCl_2*2H_2O$, neocuproine were purchased from Sigma Aldrich, potassium persulfate, HCl, methanol, $FeCl_3*6H_2O$ were purchased from Merck, and Trolox, TPTZ from Fluka.

Preparation of plant extracts

Glycerin extracts were obtained using glycerin:water ratio 60:40 (w/w). 0.5 g of grounded spelt was subjected to extraction with 5 ml solvent under various conditions as follow:

Extraction method 1(HRE) – heat reflux extraction at 60 ºC in a water bath for 4h.

Extraction method 2 (MAE) – microwave-assisted extraction for 1 min in a microwave oven (LG MS-197H) at output power 700 W for 30 s (with frequency of the waves 2450 MHz).

Extraction method 3 (UAE) – ultrasound-assisted extraction for 30 min in an ultrasonic bath (Raypa UCI 50) with frequency 50/60 Hz and power 310 W.

All obtained extracts were afterwards filtrated by Buchner funnel and were used for further experiments.

Antioxidant activity (AOA)

Total polyphenol content analysis (TPC)

TPC content was measured using a Folin-Ciocalteu assay according to the procedure described by Stintzing et al.[18] with some modifications. FolinCiocalteu reagent (1 ml) diluted five times was mixed with 0.2 ml of sample and 0.8 ml 7.5% Na_2CO_3. The reaction was performed for 20 min at room temperature in darkness. Subsequently, the absorption at 765 nm of the sample was recorded against blanc sample (prepared the same way with the addition of solvent instead of extract). The results were reported as mg equivalent of gallic acid (GAE) per gram dry weight (DW), according to calibration curve prepared with gallic acid used as a standard (0.02 - 0.10 mg).

ABTS radical cation decolorization assay

ABTS radical cation decolorization assay was performed as described by Thaipong et al.[19] with some modifications. ABTS radical was generated by mixing aliquots of 7.0 mM 2,2`azinobis (3)- ethylbenzthiazoline-6-sulfonic acid (ABTS) in dd H_2O and 2.45 mM potassium persulfate in dd H_2O. The reaction was performed for 16 h at room temperature in darkness. Prior analysis, 2.0 ml of generated $ABTS^{\bullet+}$ solution was mixed with methanol in order to obtain final absorbance at 734 nm of the working solution about 1.0÷1.1. For the assay 2.85 ml of this $ABTS^{\bullet+}$ solution was mixed with 0.15 ml of extract. After 15 min at 37 °C in darkness the absorbance at 734 nm was measured against methanol as blank. The antioxidant activity was expressed as mMTE/gDW according to calibration curve prepared with methanol solution of Trolox as standard (0.05 - 0.5 mM).

Ferric reducing antioxidant power assay (FRAP)

The assay was performed according to method, described by Benzie and Strain[20] slightly modified as follow: the FRAP reagent was freshly prepared before analysis by mixing 10 parts of 0.3 M acetate buffer (pH 3.6), 1 part of 10 mM 2,4,6- tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 part of 20 mM $FeCl_3.6H_2O$ in dd H_2O. The reaction was initiated by mixing 3.0 ml of FRAP reagent with 0.1 ml of extract. Blank sample was prepared by adding methanol instead of extract. The reaction time was 10 min at 37 °C in darkness, afterward the absorbance at 593 nm was recorded. Antioxidant activity was expressed as mM TE/gDW, according to calibration curve prepared with methanol solution of Trolox as standard (0.05 - 0.5 mM).

Copper reduction assay (CUPRAC)

Copper reduction assay was performed according to Apak et al.[21] with some modifications. The reaction was initated by mixing 1.0 ml of 10 mM $CuCl_2.2H_2O$ in dd H_2O, 1.0 ml of 7.5 mM neocuproine in methanol, 1.0 ml of 0.1 M ammonium acetate buffer (pH 7.0), 0.1 ml of extract and 1.0 ml of dd H_2O. Blank sample was prepared with adding methanol instead of extract. The reaction was carried out for 20 min at 50 °C in darkness the absorption at 450 nm was measured. The antioxidant activity was expressed as mMTE/gDW according to calibration curve prepared with methanol solution of Trolox as standard (0.05 - 0.5 mM).

Preparation of nourishing and regenerating cream formulation (NRC)

1 % of spelt extract, obtained after reflux condenser extraction was incorporated in oil in water emulsion type cosmetic formulation. The mixture was then homogenized with laboratory homogenizer.

Thermal stability of nourishing and regenerating cream formulation

Samples of the NRC formulation were placed at three temperature regimes: -10 °C; 25 °C and 40 °C. Regularly, aliquots were tested for emulsion stability and color change according the method described by COLIPA/CTFA[22]. Stable emulsion was indicated as "+", delaminated emulsion as " - ".

Skin penetration assay

The penetration ability was tested on 20 healthy female voluntaries with normal skin. An aliquot of NRC was applied to clean and dry hand skin. After 5 min the volunteer's perception of dryness was assessed as good, medium and poor using interview questionnaires.

NRC sensory test

Table 1: Total polyphenolic content (mg GAE/g DW) and antioxidant activity (mM TE/g DW) of different glycerin extracts from spelt, expressed as mean ± standard deviation. There was a statistically significant difference between groups in each column as determined by one-way ANOVA ($p<0.05$).

Extract/Assay	TPC	ABTS	FRAP	CUPRAC
HRE	0.40±0.01	5.54±0.03	1.93±0.15	11.15±0.30
MAE	0.22±0.04	3.21±0.02	1.28±0.04	4.04±0.15
UAE	0.30±0.01	4.45±0.03	1.35±0.04	7.22±0.50

Table 2: Stability of the NRC containing 1 % spelt glycerin extract.

Storage time, days/Temperature	t=40° C	t=-10° C	t=23° C
1	+	+	+
14	+	+	+
28	+	+	+
42	+	+	+
90	+	+	+

"+" – stable emulsion; "-" – unstable emulsion;

Sensory test was performed according to method described by Kanlayavattanakul et al.[23] with slight modifications. Sensory test was performed on 20 healthy female voluntaries with no respiratory disorders. An aliquot of NRC was applied to clean and dry hand skin. After 1 min odor perception of volunteers was assessed as pleasant, neutral and unpleasant using interview questionnaires.

Statistical analyses

The experiments and analysis were performed in triplicate. The results were expressed as mean ± standard deviation. Statistical comparisons were made using one-way analysis of variance (ANOVA). Statistically significant difference was defined as $p < 0.05$.

RESULTS AND DISCUSSION

It is generally thought that the yield of chemical extraction depends on the extraction time and temperature of the samples[5]. Soxhlet extraction, maceration and hydro-distillation methods have been widely used as efficient methods for bioactive compounds extraction from plant material. However, nonconventional techniques such as ultrasound-assisted extraction and microwave-assisted extractions are also of increasing demand[24]. In the present study three different extraction methods were used – heat reflux; microwave-assisted and ultrasound assisted extractions.

Antioxidant activity of spelt extracts

Total phenolic content was determined by Folin–Ciocalteu method that is based on the reducing nature of phenolics, since they may significantly contribute to the overall antioxidant activity. Total polyphenolic content (TPC) and antioxidant activity results of glycerin:water spelt extract are presented in Table 1. Three extraction methods were used: heat reflux extraction, microwave-assisted and ultrasound-assisted extractions. The investigated extract differed significantly ($P < 0.05$) in total phenolics content that is contributed to the different extraction methods used. TPC, expressed as mg equivalent of gallic acid (GAE) per gram dry weight (DW) ranged between 0.40±0.01 to 0.22±0.04 mg GAE/g DW. Best results were obtained when heat reflux extraction was used. MWA method resulted in lowest antioxidant activity. In comparison, Fogarasi et al.[25] reported similar values (0.349 to 0.593 mM GAE/g DM) for TPC of methanol/acetone extracts of whole grain samples (einkorn wheat, barley, wheat).

ABTS radical cation is another common organic radical that has been used to determine the antioxidant activity of single compounds and other complex mixtures[25]. ABTS assay measures the relative ability of antioxidant to scavenge the ABTS generated in aqueous phase, as compared with a Trolox (water soluble vitamin E analogue) standard[26]. In the present study among the three extraction methods used (Table 1) the ABTS scavenging capacity ranged from 3.21±0.02 to 5.54±0.03 mM TE/g DW. The heat reflux extracts displayed the highest value while the microwave assisted extracts – the lowest.

Another way for evaluation the antioxidant capacity of glycerin extracts is the ferric reducing antioxidant power (FRAP) assay expressed as mM Trolox equivalents per gram dry weight. This method measures the ability of antioxidants to reduce ferric iron at low pH[7]. The same tendancy was observed as ABTS method. HRE extract displayed higher capacity (1.93±0.15 mM TE/g DW) and UAE and MAE the lowest (1.35±0.04 and 1.28±0.04 mM TE/g DW respectively).

CUPRAC method is based on a redox reaction where the reactive Ar-OH groups of polyphenols are oxidized to the corresponding quinones and Cu (II)-Nc is reduced to the highly colored Cu (I)-Nc chelate showing maximum absorption at 450 nm[7]. In the present study, significant difference in results was noticed between extraction methods. For HRE extracts 11.15±0.30 mM TE/g DW was recorded compared to 4.04±0.15 mM TE/g DW for MWA extracts.

Nowadays natural ingredients increasingly enlarge their contribution in cosmetics manufacturing, due to consumers' concerns about synthetic ingredients/chemical substances. Herbal extracts are primarily added to cosmetic preparations due to several associated properties such as antioxidant and anti-inflammatory properties and tyrosinase inhibition effect[27,28]. Moreover, the stability of the colour, odour, transparency and/or active ingredients with time is also often a limiting factor[29]. However, there is a limited number of research papers in the literature regarding their usage in development of novel formulations[9].

In the present study a cosmetic product enriched with heat-reflux glycerin extract of *T. aestivum* ssp. *spelta* was prepared. Among the three extracts, HRE was prefered because of the higher biological activity. The HRE extract was characterized with pH- 5.5-6.2 and 0.7-0.8 %

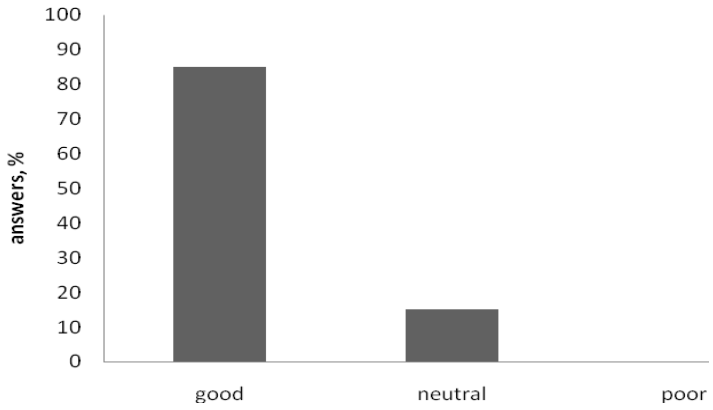

Figure 1: Skin penetration test.

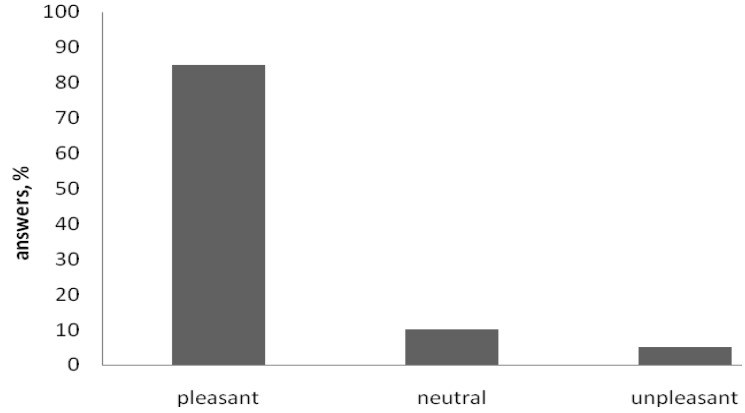

Figure 2: Sensory test.

dry content and was incorporated in oil in water emulsion type cosmetic formulation (nourishing and regenerating cream - NRC). Generally, the physical stability of disperse system affects its consistency and appearance, which reflects to product acceptance and performance. That is why emulsion and colour stabilities at different temperature over 90 days was studied (Table 2). Cosmetic cream without added plant extract was used as control sample. Lack of coalescence or loss of colour of the NRC was observed during the entire experiment. The formulation retained an uniform texture and its original pale color. The cream was stable during 90 days at all temperatures tested (-10, 23 and 40 °C).

An estimation of the penetration ability and the sensory characteristics was conducted as well. In Figure 1 are presented the results from skin penetration assay expressed as percentage of answers received. 85 % of volunteers assessed the NRC penetration as good and 15 % as neutral. No volunteer evaluated the skin penetration as poor, which indicated the good characteristic of the cosmetic cream.

In Figure 2 are presented the results from sensory assay. 17 volunteers assessed the odor of NRC as pleasant, 1 volunteers estimated the odor as unpleasant. The differences in perceptions of volunteers may be contributed to the subjective factor of the sensory test. Overall, the NRC formulation has been evaluated with positive sensory feedback (85 % of the volunteers).

CONCLUSION

The aim of the present study was to examine and compare the total phenol content and antioxidant capacity of different glycerin extracts from *Triticum aestivum* ssp. *spelta*. Three different extraction methods were used – heat reflux; microwave-assisted and ultrasound assisted extractions. Best results were obtained when heat reflux extraction was used: TPC - 0.40±0.01 mg GAE/g DW, and 5.54 ±0.03, 1.93±0.15, 11.15±0.30 mM TE/g DW according to ABTS, FRAP and CUPRAC respectively. The established total phenol content and antioxidant activity revealed the extracts of spelt as promising constituents of natural based cosmetics. Successful incorporation of 1 % glycerin spelt extract obtained under heat-reflux in nourishing and regenerating cream was performed. The cream emulsion enriched with 1 % glycerin spelt extract was stable at -10; 25 and 40 °C for 90 days. The skin penetration and sensory test results among 20 volunteers revealed good acceptance. Therefore, based on the results it could be concluded that the spelt extract is compatible with the other ingredients of the prepared cosmetic formulation and is suitable for implementation in cosmetic practice.

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